STANDARDIZED METHODS FOR THE *IN VIVO* EVALUATION OF ARTIFICIAL SURFACES*

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INTRODUCTION

At the present time it is generally agreed that new biomaterials developed for use in various artificial organs and vascular prosthetic devices should be evaluated for potential thromboresistant properties in some type of *in vivo* evaluation test. Although these materials can be implanted as "flags," "buttons," or probes in the atrial chambers of an experimental animal, there is general agreement that the most suitable method, at least at this time, for *in vivo* evaluation of biomaterials involves the fabrication of small prosthetic conduits for placement in the canine vena cava. A brief resume of the evolution of our own caval ring implant test was presented in the introductory paper for this symposium. More recently, Dr. Philip Sawyer of the State University of New York Downstate Medical Center has developed a slightly differing technique for implanting caval rings and, at the present time, these two *in vivo* methods serve as the primary means for evaluating the thromboresistance of new biomaterials.

Some of the shortcomings with this type of caval ring implant study were mentioned in our earlier presentation at this meeting. It is the purpose of this paper to discuss these standard *in vivo* evaluation techniques in more detail with particular consideration of the basic problems surrounding such a method of evaluation.

Although fabric grafts have been extensively evaluated in the experimental animal and in the broadest concept represent *in vivo* testing of biomaterials, we feel that consideration of the problem of fabric

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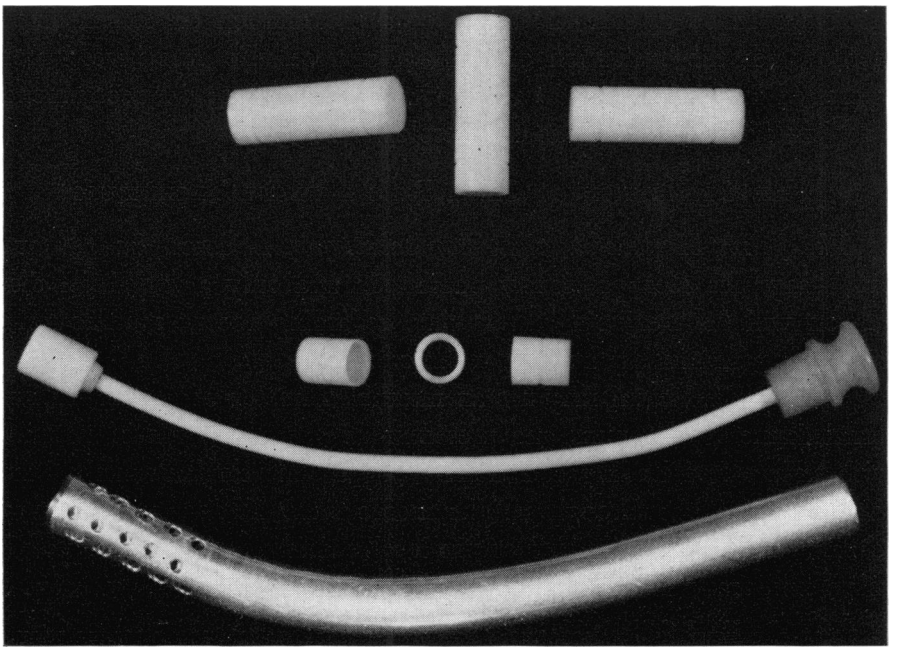
grafts would be outside the scope of a presentation on standardized methods for the evaluation of artificial surfaces. For the investigator interested in fabric graft development and evaluation, we recommend a review of the publications of Wesolowski.[1, 2]

Brief Description of Caval Ring Implant Techniques

The Johns Hopkins implantation technique. A description of our caval rings has been previously published,[3] and only a very brief discussion will be presented at this time regarding recent modifications in the ring and in the implant technique.

Our standard caval ring has an internal diameter of 7 mm., an external diameter of 8 mm., and presently an over-all length of 10 mm. with streamlined leading and trailing edges. The implantation of the ring is carried out through a right thoracotomy in dogs weighing 17 to 22 pounds. A purse-string suture is placed in the right atrium and, using temporary inflow occlusion of the cavae, the ring is placed into the inferior vena cava through a stainless steel tube (see accompanying figure), which is gently positioned in the orifice of this vessel. The use of the metal conduit simplifies the insertion procedure and eliminates any problem of contamination of the ring with tissue thromboplastin from the edge of the atriotomy. The ring is held in place in the cava by a simple 3-0 ligature and the cul-de-sac between the ring and the vein wall is obliterated by use of an external fabric wrap. Initially three to five rings of the same material are implanted in the cava for two hours. If these rings are free of thrombus after two hours, the material is considered to have moderate thromboresistance, and five similar rings are then implanted for a period of two weeks. If these rings are free of thrombus at the end of this period, the material is considered to be highly thromboresistant.

The Downstate Medical Center implantation technique. In this implantation procedure, as developed by Dr. Sawyer and his associates,[4] a polymer conduit (30 mm. in length with an i.d. of 7 mm. and an o.d. of 9.5 mm.) is implanted in the canine inferior vena cava but through a direct incision of the vein. There are external grooves 5 mm. from each end of the ring for circumferential anchoring ligatures. Ordinarily one ring of each material is placed in the canine cava for two hours and two additional rings are routinely placed for two weeks. There are two additional significant differences in this test from the Hopkins



The caval rings used by Sawyer's group at the Downstate Medical Center are depicted in the upper portion. Beneath these three rings are the caval rings and the stainless steel introducer (and stylet) used in the Johns Hopkins technique.

INTRAVENOUS CAVAL RING IMPLANT TEST:
A SUMMARY OF ITS ADVANTAGES AND DISADVANTAGES

Problem areas	*Advantages*	*Disadvantages*
A. Device stimulation	Places device in bloodstream	May be too severe a test for devices eventually placed in high velocity stream
B. Air-blood interface	Minimizes	Some devices cannot be implanted without air-blood interface (valves, grafts)
C. Fibrinolysis	Minimizes	Fibrinolysis probably important on many devices (valves, balloons, pumps)
D. Turbulence and stasis	Minimizes	Many devices reside in turbulent and static stream (valves, balloons, pumps)
E. Endocardial—prosthetic junction	Minimizes	Many devices have endocardial-prosthetic suture line (valves, grafts, art. hearts)
F. Miscellaneous	—	Cannot evaluate highly flexible materials as rings
		Cannot monitor dynamic changes in thrombus

implant procedure. The first is that the animals are approximately twice the size of the animals used in the Hopkins test and, with rings of the same internal diameter, this means that the volume of venous return and thus the velocity of blood flow through these rings will be significantly higher. The other major difference in the two-ring implant techniques is the fact that in the Downstate test an external fabric wrap is not used to obliterate the prosthesis-vein wall cul-de-sac at each end of the ring. This may or may not be of importance in the ring study.

General Information Obtained to Date Using the Caval Ring Implant Tests and a Comparative Study of Materials Evaluated in Each Type of In Vivo Test

During the past 10 years dozens of new biomaterials with potential thromboresistant properties have been screened by use of the caval ring implant tests. Three years ago the Artificial Heart Contract Office (currently the Medical Devices Development Program) selected the Hopkins' ring test as a standard method for screening new materials developed by 15 different contract groups. This past year they have also submitted a number of the contract materials to Dr. Sawyer's laboratory for evaluation of thromboresistance.

On the basis of the biomaterials that have been evaluated in our laboratory during the past 10 years, we feel that most of the artificial surfaces with significant thromboresistant properties fall into three categories: heparinized surfaces, surfaces having anionic radicals or imposed negative electrical charges, and surfaces of relatively inert materials.

The various surfaces in each of these three categories were considered in detail in a recent presentation[5] and will not be further discussed at this time.

During the past year, with the more extensive use of Dr. Sawyer's caval ring implant technique, it has been interesting to compare the results of biomaterials evaluated by each of these standard *in vivo* methods. In comparing the results obtained by the Downstate investigators with our own results in the evaluation of various biomaterials the following conclusions can be made at this time. Plain polymer surfaces such as polycarbonate show no evidence of thromboresistance in either study; all rings were severely thrombosed after two hours in the vena cava. Similarly, there is excellent correlation in both labora-

tories with materials that are felt to have outstanding thromboresistance.

With a number of materials that seem to have only moderate thromboresistance, the correlation is not particularly good in that a greater degree of thrombus is usually observed in the Hopkins rings than in the Downstate rings. Thus materials that have been related as having moderate but not significant thromboresistance by Dr. Sawyer's group have frequently been given a poor rating by our group. The greater degree of patency in the Downstate rings may well be related to the higher velocity of caval blood flow which in turn results from the use of larger test animals.

In summarizing, then, a comparison of these two standard *in vivo* evaluation methods, it appears that they correlate extremely well with regard to the very poor and the very good biomaterials, and for surfaces with intermediate thromboresistance the Hopkins test is more severe.

The Advantages and Disadvantages of Standard Caval Ring Implant Tests

It would appear now, after 10 years of experience with the caval ring implant tests, that there are a number of advantages of these two tests over other evaluation methods, but it is true that there are also some significant disadvantages with this type of biomaterial testing.

We have elected to categorize the problem areas of biomaterial evaluation into the six subheadings listed in the accompanying table, and we now present what we feel are the advantages and disadvantages of the caval ring tests in each of these areas.

Device simulation. Certainly when comparing the caval ring technique with *in vitro* screening tests and *ex vivo* blood cell systems, the ring study provides a significant advantage as far as device simulation. With this type of evaluation method, a prosthetic device is actually placed within the blood stream and it tests the biomaterial much as it might be used in a more complicated intravascular prosthesis. A disadvantage, however, may be that the caval ring implant is too severe a test for some biomaterials that would eventually be used in a high-velocity blood stream. Certainly a material for use as an arterial conduit would not have to pass the rigorous requirements of the caval implant test.

Air-blood interface. Again, when comparing the ring implant test with *in vitro* tests, the former has the distinct advantage of almost

completely eliminating the air-blood interface, which can certainly overshadow the physical and chemical reactions that occur at the blood-prosthetic interface. On the other hand, it should be remembered that a number of prosthetic devices cannot be implanted without the problem of the air-blood interface. For example, when a prosthetic valve is placed in the mitral position the left heart is reasonably dry for the most part, but there is always a certain amount of blood in contact with the prosthetic surface during the implantation. Thus, there is an alternative environment of blood and of air and blood during the implantation of such a device. The same exposure to blood and air may occur with the implantation of other prosthetic devices such as vascular grafts.

Fibrinolysis. One of the primary disadvantages of implanting "buttons," "flags," or probes in the right atrium for biomaterial screening is that fibrinolysis can significantly affect the deposit of thrombus on these test devices. In our experience with these right atrial screening techniques, the test device may have a massive thrombus within four hours and by 24 hours after implantation the thrombus can be completely lysed. On the other hand, if the surface of the caval ring is not particularly thromboresistant, the ring will usually thrombose within two to four hours and there will be no longer blood flow over the thrombus, thus minimizing the fibrinolytic effect.

The disadvantage of the caval ring test in this particular problem area is the fact that fibrinolysis probably plays a very important role in minimizing significant thrombus build-up on many prosthetic devices. The fibrinolytic system must be extremely important in the long-term function of prosthetic valves, intra-aortic balloons, and cardiac assist pumps.

Turbulence and stasis. The caval rings, because of their streamlined configuration, tend to minimize turbulence and stasis, two factors which greatly enhance the deposit of thrombus on prosthetic surfaces. This would appear to be a significant advantage of this type of testing device, and yet many cardiovascular prosthetic devices either reside in or generate a turbulent or static blood stream, i.e., prosthetic heart valves, intra-aortic balloons, and cardiac assist pumps.

Endothelial-prosthetic junction. Again, because of the streamlined leading and trailing edges of these caval conduits, the junction of the prosthesis and the vein wall is reasonably smooth. This is probably even

more true of the Hopkins ring test because of the use of an external fabric wrap in this study. Also, there is no suture line to contend with, which, of course, because of trauma to the endothelium, greatly enhances thrombotic deposit. Since many of the prosthetic implants with which, of course, because of trauma to the endothelium, greatly enficial hearts require the use of suture anchoring at the endothelial-prosthetic junction, the caval ring does not then simulate the problem that would occur with these devices.

Miscellaneous problem areas. There are probably a number of additional disadvantages of the caval ring implant tests, but we shall discuss only two. The first is that the caval ring study lends itself only to the evaluation of rigid or semirigid materials. It is not suitable for the evaluation of membranes or highly flexible materials that would collapse if placed as a ring in the vena cava. We have been able to implant some highly flexible silicone rubber rings and hydrogel rings with success, but with extremely deformable materials a suitable ring study cannot be carried out. It would appear at this time that for these deformable materials, the best method of evaluation, if possible, would be the implantation of the material as a venous graft using a suture anastomosis.

The final disadvantage of the caval ring implant test to be enumerated in this presentation, and probably the most significant, is the fact that there is not a suitable way of monitoring the dynamic changes in thrombus deposit and dissolution on the surface of the ring. This particular factor was briefly considered in our opening presentation for this symposium, and it probably is such a significant problem that we should give a much more concerted effort to the development of suitable *ex vivo* screening tests in which, at least with transparent materials, the buildup and lysis of a thrombus could be observed.

It is important to emphasize at this point that we do not feel that there is a significant problem with lysis and embolization when the caval ring becomes completely occluded. This, in our experience with hundreds of ring implants over the last 10 years, seems to be a very rare occurrance. We do feel, however, that caval rings constructed of materials of moderate thromboresistance may develop a 1- to 2-mm. thick laminar thrombus over four to 12 hours, and then with continued flow through the conduit, permit subsequent lysis of the thrombus and even particulate embolus from the ring. We do not believe that Dr. Bert K. Kusserow's studies[6] on embolization rings placed in the aorta above

the kidney are completely applicable to the caval ring study, in that in his technique rings are anchored in the high velocity aortic stream by a single circumferential ligature with a resulting cul-de-sac between the internal aortic wall and each end of the ring. With this opportunity for high turbulent flow, we should anticipate that even the most thromboresistant rings would be the site of deposit of thrombus, particularly between the ring and the aortic wall. We hope that Dr. Kusserow will continue with his embolic studies, but implant streamlined rings in the aorta with an external fabric wrap to eliminate the significant factor of turbulence.

It may be that although the build-up and lysis of thrombus within a caval ring cannot be visually observed, the size of the thrombotic deposit might be electronically monitored using a thermo-couple system. This type of heat-transfer device has been previously used for the monitoring of thrombus build-up and lysis on atrial probes.[7]

In summary, the caval ring implant procedures appear to provide a number of advantages over *in vitro* screening methods and over other types of *in vivo* techniques. There are, however, some distinct disadvantages with this method of biomaterial evaluation; these are briefly summarized in the table shown. It would appear at this time, then, that although the caval ring tests are the mainstay of the *in vivo* evaluation procedures, and for some time to come may serve a very useful purpose, a concerted effort should be made to develop more suitable biomaterial screening techniques. These newer screening methods will have to take into account two major factors. First is the ability of the screening test to simulate more closely the environmental situation in which the biomaterial eventually finds itself. In other words, the test that screens a material for aortic balloon construction may not be a reasonable test for a polymer that will be used in the housing of a prosthetic valve. The other factor is the need to further develop *ex-vivo* tests so that the dynamic nature of the thrombus deposit can be better monitored.

Conclusions

At the present time the caval ring implant tests appear to be the best way of screening biomaterials for potential thromboresistance. The use of the caval implant tests over the last 10 years has provided considerable information in determining which materials might be more

suitable for the construction of cardiovascular prosthetic devices. There appear to be a number of advantages of the caval ring test over *in vitro* screening methods and other *in vivo* methods, but there are also some significant disadvantages. These relate primarily to the dissimilarity of the caval prosthetic conduit and the actual fabricated prosthetic device which may be implanted in the venous system, arterial system, or within the heart. The other major disadvantage of the caval ring technique is the fact that the dynamic process of thrombus deposit and lysis cannot be readily monitored.

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